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CERTIFICATE OF MAILING

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DECLARATION UNDER 37 CFR 1.132

Sir:

I, Jennifer L. Schmitke, of 476 Shawmut Ave. #5, Boston, Massachusetts 02218, am an inventor of the above identified application.

The invention which is described in the above identified application results from substantial experimentation that necessitated the selection of a formulation that achieves serum insulin levels similar to that achieved by injectable insulin, good to excellent bioavailability, good to excellent physical stability and good to excellent manufacturability. The formulation of Claim 1 unexpectedly accomplishes all of these

objectives. Below, I describe experiments that I conducted or were conducted under my supervision or direction.

1.0 Background

AIR Human Insulin Inhalation Powder (HIIP) is a spray-dried powder comprised of human insulin (BHI), sodium citrate, and DPPC (1, 2–Dipalmitoyl-*sn*-Glycero-3-Phosphocholine The HIIP manufacturing process involves two (2) liquid feed streams: an ethanol-based solution containing the surfactant DPPC, and an aqueous solution containing BHI and citric acid monohydrate. During the batch production process, the organic solution and the aqueous solution are continuously pumped to the spray dryer at a controlled 60/40 (organic/aqueous) volumetric ratio. The feed streams are individually pre-heated to ~50°C and then combined in an in-line static mixer just before entering the atomizer for the spray dryer.

The solubility of the combined organic/aqueous feed stream for the HIIP process was investigated to ensure that no solids would precipitate or "crash out" once the individual phases were combined. Any solids in the combined feed stream to the spray dryer could significantly impact solution atomization, and subsequently affect the aerosol performance of the HIIP product.

2.0 Objective

The primary objective of this study was to identify maximum solids concentrations for different compositions of Human Insulin Inhalation Powder (HIIP) formulations. The study characterized the varying solubility range for a combined 60/40 vol.% organic/aqueous feed solution at 50°C.

The secondary objective was to determine if holding the individual organic and aqueous phases stirring at room temperature over a period of eight hours (typical duration for a

manufacturing campaign) prior to combination affected the solubility of the 60/40 vol.% organic/aqueous feed solution.

3.0 Testing Methods

3.1 Solubility vs. Concentration and Composition

Several sets of solutions were prepared (Reference Appendix A for solution preparation procedures) at different solids concentrations and compositions as shown in the table below. All solutions were 60/40 vol.% ethanol/water.

Table 1: Compositions and Concentrations of Test Solutions

Solution Label	Wt.% Insulin	Wt.% DPPC	Wt.% Sodium Citrate	Total Solution Concentration (g/L)
10% Insulin	10	80	10	5
15% Insulin	15	75	10	5, 10, 15
20% Insulin	20	70	10	5, 10, 15
25% Insulin	25	65	10	15, 25
30% Insulin	30	60	10	8, 10, 11, 12, 15, 20
30% Insulin	30	60	10	25, 30, 40, 50

The organic and aqueous phases of each test solution were prepared at room temperature and then placed in a 50°C Water Bath for 30 minutes to an hour. The individual phases were combined once they reached the 50°C equilibration temperature. The combined solution was returned to the water bath, and then monitored visually until any particles were observed to "crash out" of solution.

3.2 Solution Solubility vs. Time

To investigate whether "aging" of the individual organic and aqueous phases could affect the solubility of the combined solution, aqueous and organic solutions were prepared and stirred at room temperature over an eight-hour period. The 60/30/10 weight percent DPPC/insulin/citrate formulation (i.e, 30% HIIP formulation) at a total solids concentration of 15 grams per liter was selected for this study. The preparation of these solutions is detailed in experiment 04-xxx-357-181 (notebook 357, page 181).

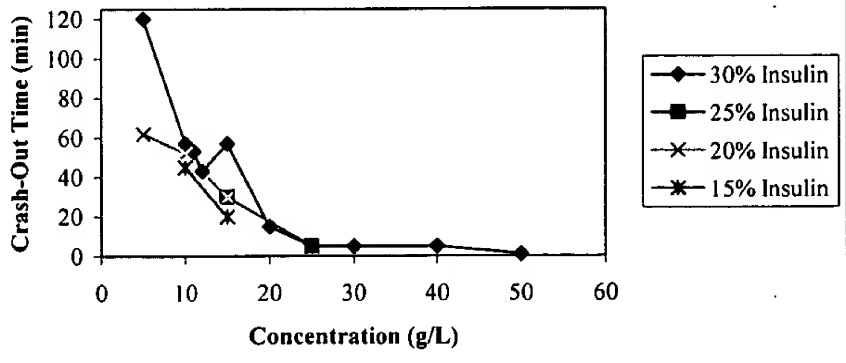
At times of 0 (fresh feed), 2, 4, 6 and 8 hours, a sixty milliliter (60 ml) sample was drawn from the organic phase and a forty milliliter (40 ml) sample was drawn from the aqueous phase. The aqueous and organic samples were placed into a 50°C Water Bath for 15 minutes to achieve temperature. The samples were then combined and returned to the water bath. The combined solution was monitored visually until particles were observed to "crash out" of solution.

4.0 Results and Discussion

4.1 Solubility vs. Concentration and Composition

Figure 1 shows "crash-out" time as a function of solids concentration and percent insulin composition. The higher the total solids concentration, the faster solids were observed to come out of the combined 60/40 vol% org/aq. solution. Although the 30% insulin formulation was not limited by solubility until it reached a concentration of 50 g/L, rapid

Figure 1
Concentration vs. "Crash-Out" Time



"crash-out" times (i.e., 5 minutes or less) were observed at concentrations of 25 g/l and higher.

Figure 2
Composition vs. "Crash-Out" Time

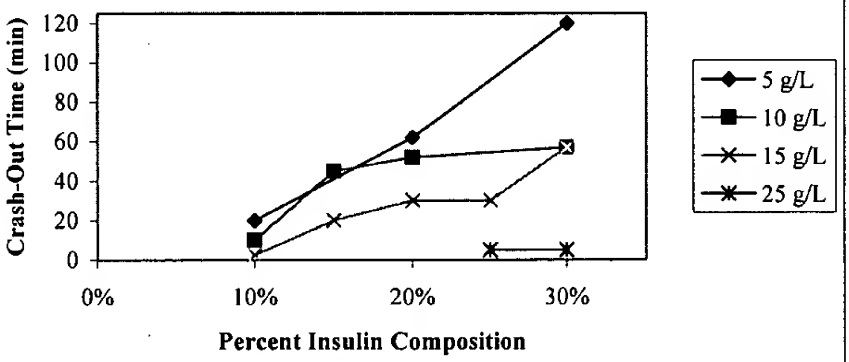


Figure 2 shows that higher insulin content solutions appear to stay in solution longer (i.e., have higher "crash-out" times). This could be because higher insulin content translates to a lower DPPC content (as seen in Table 1).

Figure 2 also shows that the 10% insulin formulation has rapid "crash-out" times (i.e., 5 minutes or less) for concentrations above 10 g/l. This observed drop in solubility as concentration increases makes the 10% insulin formulation an unattractive "low-load" insulin formulation from a process manufacturing point of view. The limits in feed solution solubility for the 10% insulin formulation would hence limit the possible solids throughput in the manufacturing scheme.

In other words, the 10% insulin formulation experienced rapid "crash-out" times with a DPPC concentration of 4.0 g/l, an insulin concentration of 0.5 g/l and a citrate concentration of 0.5 g/l. However, the 30% insulin formulation did not "crash-out" even at concentrations of 20 g/l total solids, resulting in a DPPC concentration of 12.0 g/l, an insulin concentration of 6 g/l and a citrate concentration of 2 g/l. Clearly, the solubility

of the insulin formulation is not dictated solely by DPPC solubility. Such a dramatic improvement in manufacturability could not have been predicted.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon

Jennifer L. Schmitke

Appendix A: General Solution Preparation

General solution preparation procedures for organic and aqueous insulin feed solutions are outlined below.

Total feed solution volume = **V**

Total solids concentration = **C**

FORMULATION = weight percents of DPPC/Insulin/citrate as summarized in Table 1.

ORGANIC PHASE SOLUTION PREPARATION:

1. Measure out 200 proof ethanol in amount that is 60 vol% of total feed solution, **V**.
2. Measure out DPPC in amount that is DPPC wt.% of total solids concentration, **C**.
3. Add the DPPC into the ethanol and mix with a magnetic stir bar until the DPPC has completely dissolved into solution.

AQUEOUS PHASE SOLUTION PREPARATION:

1. Measure out Sterile Filtered Water in amount that is 40 vol% of total feed solution, **V**.
2. Measure out citric acid monohydrate in amount that is 8.4 wt.% of total solids concentration, **C**.
3. Add the citric acid monohydrate into the sterile filtered water and mix with a magnetic stir bar until the citric acid has completely dissolved into solution.
4. Calibrate a pH meter from pH range pH= 4 to pH=7. (Check reading in pH=2.0 buffer, and re-calibrate if pH reading is not pH=2 ± 0.1).
5. Measure the initial pH of the citrate buffer. If the pH is greater than 2.5, adjust the pH to pH=2.5 ± 0.05 with the addition of 1.0 N HCl (hydrochloric acid)
6. Measure out insulin in amount that is Insulin wt.% of total solids concentration, **C**.*
7. Add insulin into the citrate buffer and mix with a magnetic stir bar until the insulin has completely dissolved into solution.

8. Measure the pH . Adjust the pH = 6.7 ± 0.05 with the addition of 1.0 N NaOH (sodium hydroxide).